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Properties of a Commercial Fungal Pectase Preparation [1]

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Introduction

Since the discovery of the action of pectase (pectinesterase) by Fremy in 1840, many workers (1–4) have reported on the properties of pectase occurring in the roots, leaves, and fruits of the higher plants. Properties of fungal pectase, however, have received relatively little attention. As a rule, pectinase (polygalacturonase) and pectase occur together in fungi, and pectase was probably overshadowed by pectinase, which generally has much greater activity (5).

Recently McColloch and Kertesz (6) compared the behavior and action of pectases from higher plants with the pectase in commercial pectinase of fungal origin. Fish and Dustman (7) determined the optimum pH for pectase in Pectinol A, a commercial preparation of pectinase. These investigations indicated that fungal pectase has a tolerance for a lower pH than pectases from higher plants. This tolerance should enable its use in fruits for gels and aspics, for which uses the pectase of higher plants is not suited (8).

Recently a fungal pectase, essentially free of pectinase, was made available commercially.[3] The investigation reported here was undertaken to determine the properties of this enzyme, to compare it with pectases from higher plants, and to determine its potential usefulness in the preparation of fruit gels.

[1] Report of a study made under the Research and Marketing Act of 1946.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

[3] Pectinesterase No. 4, manufactured by the Rohm and Haas Company, Philadelphia, Pennsylvania.

Materials and Methods

Preparation of Pectase

Twenty ml. of distilled water was added per gram of dry enzyme, the mixture allowed to extract for 1 hr. at room temperature, and then was filtered. Water extracts were used in preference to dry enzyme in order to facilitate dispersal of the enzyme in the reaction mixtures.

Preparation of Pectin

The apple pectins were purified by the method used by Hills and Mottern (4). The pectin samples were characterized by the following properties: Pectin 1: moisture, 6.92%; ash, 0.48% (moisture-free basis); CH_3O (Zeisel), 9.18%. Pectin 2: moisture, 11.6%; ash, 0.66%; CH_3O (Zeisel), 8.48%. The polyuronide content probably was in the range of 75–85% which is usual for most apple pectins purified by this method. All analyses were made on ethanol-free samples. Since the properties of the pectin samples are similar, when the supply of Pectin 1 was diminished, Pectin 2 was substituted in the substrate without affecting the data.

Method of Pectase Determination

A modification of the Kertesz (9) titration method was adopted. In the standard procedure used, the substrate contained 0.4% pectin, 0.05 *M* sodium chloride, and 0.002 *M* sodium oxalate. A 100-ml. aliquot of substrate was measured into a 250-ml. beaker, the reaction mixture was heated to 30 ± 1°C. and maintained at that temperature in a water bath. An electrically driven glass stirrer was used to agitate the solution; pH was determined with a pH meter equipped with extension leads. The pH of the reaction mixture was adjusted to approximately 7.5 with 0.5 *N* sodium hydroxide, and after the enzyme solution was added the pH was adjusted to exactly 7.50. ess otherwise stated, the mixture was then quickly adjusted to pH 3.5 with 0.5 *N* sulfuric acid, and the amount of acid required was recorded. The reaction was permitted to proceed for exactly 30 min., at which time the pH was quickly adjusted to 7.5 with 0.5 *N* sodium hydroxide. All adjustments of pH were made in a matter of seconds. From the amount of alkali used, the activity of the enzyme was calculated.

Experimental

Rate of Deësterification

The rate of deësterification was measured by allowing the enzyme to react for different periods of time. It was determined that deësterification can be calculated as either a first- or zero-order reaction for at least the first 40% of hydrolysis. As a matter of convenience, our data have been calculated on the basis of an apparent zero order. Similar anomalous kinetics were observed in the deësterification of pectin by tomato pectase (4). Figure 1 shows the rate of deësterification by fungal pectase.

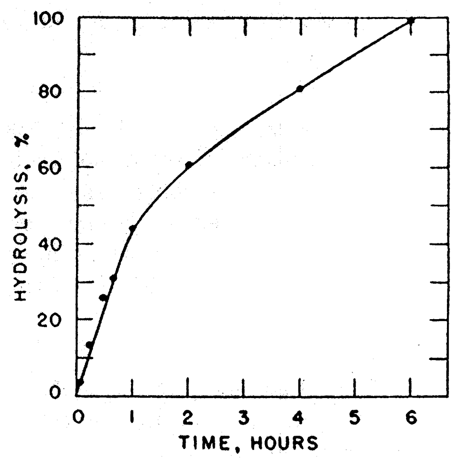

FIG. 1. Rate of deësterification.

By measuring the pectase activity within the region of the apparent zero-order reaction, we satisfy the test for a valid method of measurement, in that the rate of activity is proportional to enzyme concentration over a wide range of added enzyme. Pectase activity is expressed as the zero-order velocity constant, where K is in units of milliequivalents of bonds hydrolyzed per minute per gram of dry

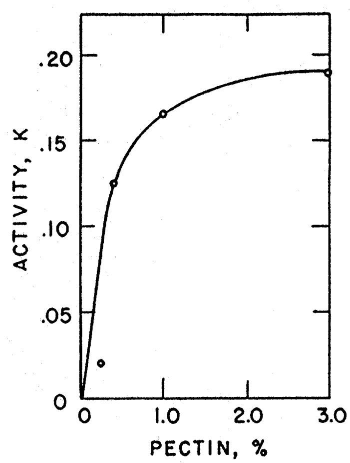

FIG. 2. Enzyme activity as a function of substrate concentration.

enzyme. Three quantities of enzyme, 0.05, 0.20, and 1.00 g. gave, respectively, the following K values: 0.130, 0.125, and 0.135.

Effect of Pectin Concentration

By measuring the rate of deësterification of substrates containing concentrations of pectin ranging from 0.2–3.0%, the relation of pectase activity to pectin concentration was determined. In this range, pectase activity increased as the concentration of pectin increased (Fig. 2). With 0.4% pectin, the concentration used in the standard substrate, pectase activity was 69.4% of that with 3% pectin.

Effect of pH on Pectase Activity

The relationship of pH and fungal pectase activity was determined in the presence of 0.05 M NaCl. The data (Fig. 3) indicate that the range of activity extends from pH 2.0 to pH 6.5 with the optimum at pH 5.0. The optimum region is fairly broad, however, extending from pH 4.0 to 5.5.

While the observations of Fish and Dustman (7) and McColloch and Kertesz (6) on the pectase in commercial pectinase are not identical with ours, the variances can be attributed to the differences in conditions under which their enzyme was studied and to the difference in our enzyme preparations. Their data are in agreement with ours indicating that fungal pectase differs from pectases from higher plants.

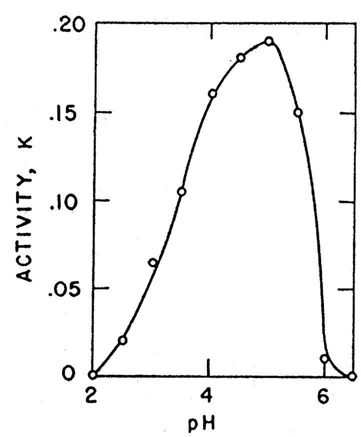

FIG. 3. Effect of pH on pectase activity.

Effect of Mono- and Divalent Cations on Pectase Activity

As has been shown by Lineweaver and Ballou (1), MacDonnell *et al.* (2), Hills and Mottern (4), and other workers, activities of plant pectases are influenced markedly by mono- and divalent cations.

To determine the cation effect on fungal pectase, activity was measured at pH 3.5 and pH 5.5 in the presence of different concentrations of NaCl. The results are shown in Fig. 4. At pH 3.5, optimum activity was obtained in the presence of 0.5 *M* NaCl, and the increase in activity extended over a broad range. At pH 5.5, optimum activity was obtained in the presence of 0.2 *M* NaCl. Lower concentrations of NaCl are required to activate fungal pectase in the region of its optimal pH than would be required at a lower pH. It should be noted that small amounts

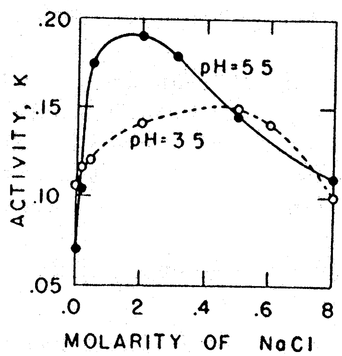

FIG. 4. Effect of NaCl on pectase activity.

of cations were contributed by the ash of the pectin substrate, by the pectase solution and by the alkali used for pH adjustments. The ion concentration of the substrate was calculated to be approximately 0.004 *N*, and that contributed by the enzyme solution, as determined from conductivity data and calculated as KCl, was found to be approximately 0.003 *N*.

In measuring the effect of $CaCl_2$, a substrate containing 2% purified apple pectin was used with 0.1 *N* sulfuric acid and sodium hydroxide for the titrations. Activity was measured at pH 3.5 and 5.5 in the presence of various concentrations of $CaCl_2$. Results of this experiment are shown graphically in Fig. 5. At pH 3.5, the enzyme is activated by

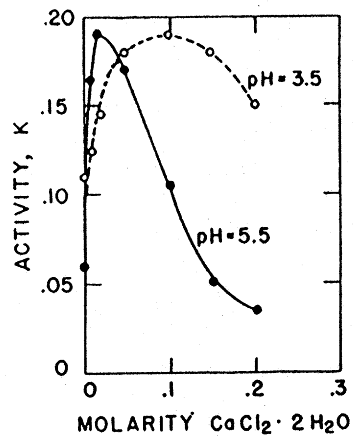

FIG. 5. Effect of $CaCl_2 \cdot 2H_2O$ on pectase activity.

concentrations of $CaCl_2$ ranging from 0.01 to 0.1 M, the activity decreasing with higher concentrations of the salt. At pH 5.5 the enzyme is most active in the presence of 0.01 and 0.02 M concentrations of $CaCl_2$. Higher concentrations of the salt have an inhibiting effect. The data indicate that fungal pectase is more sensitive to divalent cations than to monovalent cations and that smaller quantities of the former are required to achieve maximum activity.

Variation of Pectase Activity with Temperature

Shorter reaction times were used at higher temperatures to confine the reaction within the initial 40% deësterification. Table I shows the increase in rate of the enzyme reaction with increase in temperature, expressed as the Q_{10} value. The data indicate that fungal pectase differs slightly from higher plant pectases in its temperature coefficient.

TABLE I

Temperature Coefficient of Fungal Pectase Deësterification of Pectin

Temperature interval °C.	Temperature coeff. Q_{10}
30–40	1.46
40–50	1.40
50–60	0.94

Inactivation of Pectase by Heat

The standard enzyme extract was used. The pH was 3.5, and the reaction time in each case was 30 min. There was 50% reduction in activity at 58.5°C. and complete inactivation at 62° (Fig. 6). Fungal pectase is apparently more sensitive to temperature than tomato pectase (pH 6.5) which is 50% inactivated at 62° (4). The temperature

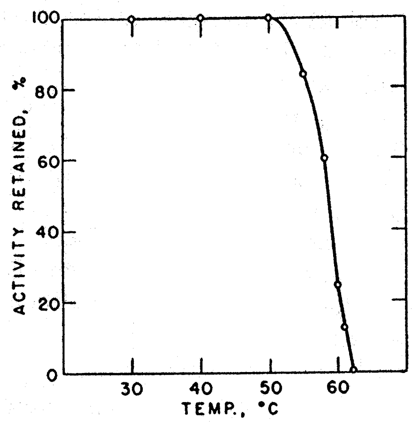

FIG. 6. Inactivation of fungal pectase by heat when heated for 30 min. at pH 3.5.

required for 50% inactivation of fungal pectase is higher, however, than that required for orange pectase (2) or Pectinol PM (6).

Stability of Fungal Pectase Extracts

To determine the rate of deterioration of stored fungal pectase extracts, samples were held at 5–6° and at 23°C. The pH was 6.25. The extract was filtered through a Seitz filter before being stored at 23°C. There was no loss of activity of the enzyme during a 2-week period at either temperature.

Pectinase

To determine whether fungal pectase was free of pectinase, the reduction in viscosity of a 2% pectic acid substrate was measured at different time intervals with an Ostwald-Cannon-Fenske viscometer (No. 200) at 30.5°C. By comparing the reduction in viscosity by the pectase extract and by a sample of Pectinol 100 D (0.1 mg./ml.), it was determined that fungal pectase is essentially free of pectinase. The conversion of our data into clarification units[4] gives a value of 0.3 of a pectinase unit per gram of dry pectase as compared to 10,000 units/g. of Pectinol 100 D.

Discussion

This preparation of fungal pectase differs from the higher plant pectases in its pH relationship, in its response to mono- and divalent cations, and in its thermal behavior. Its range of activity, which extends from pH 2.0 to 6.5 in the presence of 0.05 *M* NaCl, differs strikingly from the behavior of tomato, alfalfa, orange, and tobacco pectases, which are inactive below pH 4.0. The tolerance of fungal pectase to low pH suggests the practicability of its use in the gelling of fruits at pH 3.0–4.0 or vegetable aspics at pH 3.5–5.5. A combination of fungal pectase and of a higher plant pectase would enable the use of a mixture of these enzymes over a wide pH range.

In view of the possible use of fungal pectase in the preparation of fruit gels and vegetable aspics, many of our studies were carried out at pH 3.5 and 5.5, although optimum activity is at pH 5.0. Formulation studies will be reported at a later date.

Acknowledgments

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Summary

1. The commercial fungal pectase used was essentially free of pectinase.

2. Deësterification of pectin was an apparent zero-order reaction for the first 40% of hydrolysis.

3. The enzyme was active within the range of pH 2.0–6.5 with optimum activity at pH 5.0.

4. Optimum salt concentrations varied with pH. At the optimum, smaller quantities of NaCl and $CaCl_2$ were required for full activation. The pectase was more sensitive to calcium than to sodium ions.

[4] Method used by Rohm and Haas Company, Philadelphia, Pa.

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5. The Q_{10} value was 1.46 between 30° and 40°C. Inactivation of the enzyme became apparent above 50°.

6. The enzyme, in solution at its natural pH of 6.25, remained stable through a 2-week period at 5–6°C. and at 23°C. A temperature of 62° completely inactivated the enzyme in 30 min. at pH 3.5.

7. With respect to the above properties, fungal pectase differs quantitatively from higher plant pectases.

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